Q-238/453 Desulfovibrio vulgaris Hildenborough biofilm formation by wild type, the mega-plasmid deletion strain, and a *fur* deletion mutant

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ESPP Environmental Stress Pathway Project

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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ABSTRACT

D. vulgaris forms biofilms under stress and nutrient limitation conditions. Two different assay methods were used to estimate the biofilm formation. The results indicate that *D. vulgaris* formed more biofilm in late stationary phase and at temperatures from 24 to 30 C when compared to earlier growth phases or higher temperatures. The *fur* deletion mutant (JW707) formed more biofilm and at an earlier growth stage than the wild type. The mega-plasmid deletion mutant (JW801) had similar biofilm forming ability as the wild type. In nutrient limitation studies, limiting the electron donor with excess electron acceptor (sulfate) favored biofilm formation. Biofilm formation decreased when the medium pH decreased from 7 to 5. This may simply be a result of the lower cell densities attained at acid pH. Addition of sugars (glucose, galactose, mannose, or gluconate) did not significantly effect the biofilm formation; however, addition of sugars to acid pH (pH 5.5) medium completely inhibited the cell growth and biofilm formation. Finally, when the biofilm formation was assayed by the content of the biomass which was attached to the bottom of test tubes, it appeared that formation was influenced by the iron concentrations (FeCl2) in the growth medium.

RESULTS

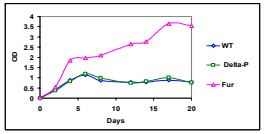
Table 1. Temperature effect on biofilm formation by *D. vulgaris* WT: Lower temperature appears to favor biofilm formation

Supplement	24 C (254 hr)		30 C (110 hr)		37C (110 hr)	
	Protein[a] µg/ml	Biofilm[b] OD	Protein µg/ml	Biofilm OD	Protein µg/ml	Biofilm OD
None	84	0.988	101	0.84	101	0.722
200 µM Norspermidine	98	1.631	112	1.51	ND[b]	ND
200 µM Sermidine	99	1.518	119	0.81	ND	ND

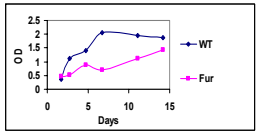
[a]Protein of the growing culture
[b]The cultures (10 ml) were removed from the Hungate tubes. The tubes were rinsed gently with water 3X and stained with 0.05% crystal violet (10 ml) for 40 min. After staining, the glass tubes were rinsed with water 3X. The biofilms were dissolved in 10 ml alcohol: acetone (80:20 v/v) for 30 min and ODs read at 600 nm.
[b] ND, Not determined

Figure 1. Biofilm formation[a] by *D. vulgaris* wild type, mega-plasmid deletion (delta-P) and Δ*fur* mutant under undisturbed and agitated conditions

I. Undisturbed



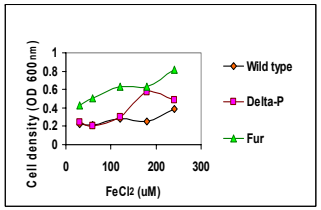
II. Aggitated by hand



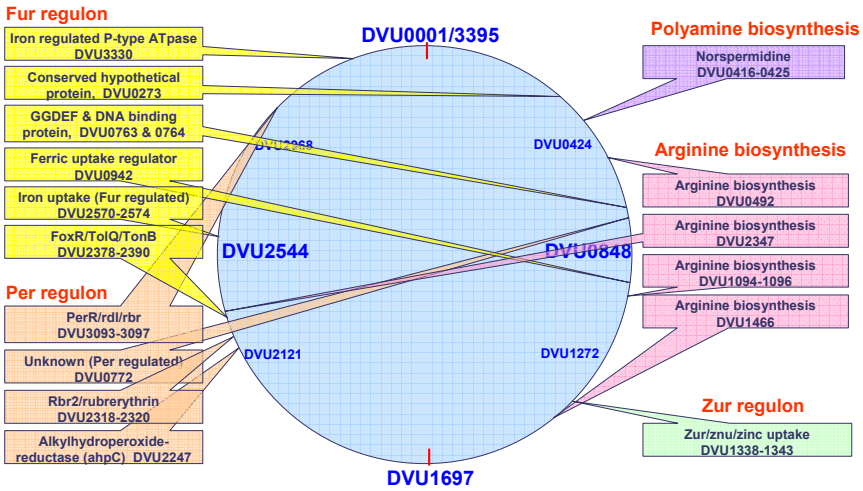
[a]Biofilm measured as crystal violet stainable material

Figure 2. Effects of iron concentrations on biofilm formation*

Assay for biofilm cell density or biofilm protein measures cells adherent to bottom of tube.



PREDICTED REGULONS TESTED



Putative arginine and polyamine biosynthetic pathway

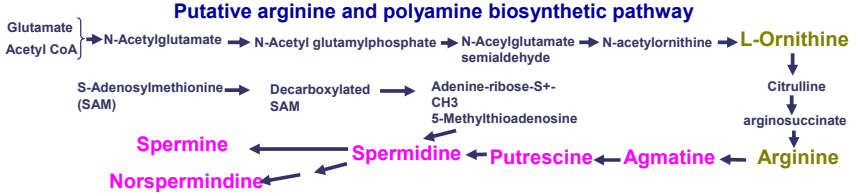


Table 2. Effect of Arginine pathway and polyamines on biofilm formation on the wild type, Δ*fur*, Δ*perR*, and Δ*zur* mutants

Biofilm on glass[1]	Undisturbed cutlure[2]				Agitated culture[3]			
Additions	Wild Type	ΔFur	ΔPerR	ΔZur	Wild Type	ΔFur	ΔPerR	ΔZur
	OD of crystal violet stain				OD of crystal violet stain			
None	0.43	0.48	0.70	0.44	2.4	2.4	1.4	1.6
Arginine	0.78	0.46	0.81	0.48	2.0	2.2	1.3	1.4
Ornithine	0.62	0.71	0.64	0.34	2.5	1.4	1.2	1.3
Agmatine	0.52	0.60	0.61	0.42	--[4]	--	--	--
Putrescine	0.59	0.50	0.61	0.35	2.5	2.7	1.3	1.1
Cadaverine	0.44	0.56	0.80	0.36	--	--	--	--
Spermidine	0.96	0.81	0.95	0.36	3.7	3.3	3.4	1.4
Norspermidine	1.01	1.05	1.44	0.40	2.9	3.1	3.9	2.3
Spermine	1.12	0.72	0.87	0.72	--	--	--	--
	Biofilm Protein (ug/ml)				Biofilm Protein (ug/ml)			
None	11	95	97	93	19	17	17	99
Arginine	119	92	116	89	33	15	37	91
Ornithine	160	102	136	87	58	21	43	107
Agmatine	121	90	107	77	--	--	--	--
Putrescine	114	95	102	89	34	18	28	81
Cadaverine	104	87	88	79	--	--	--	--
Spermidine	90	84	84	53	17	23	30	135
Norspermidine	78	72	97	105	20	13	21	120
Spermine	69	60	57	50	--	--	--	--

[1]Biofilm was assayed with the standard cyrstal violet stain (glass tube wall) and protein assay (cells attached to tube bottom)
[2]Cultures were grown on lactate-sulfate medium (60 mM lactate and 30 mM sulfate) at 30 C for 186 hours undisturbed
[3] Agitation was by inversion
[4] – Not determined

Table 4. No dramatic effects of nutrient limitations on biofilm formation

Nutrient limitation	37 C (224 hours) Protein µg/ml	37 C (224 hours) Biofilm OD	30C (280 hours) Protein µg/ml	30C (280 hours) Biofilm OD
A. Positive Control	126	1.28	149	1.21
B. NH^{4+} limited	90	1.65	51	1.29
C. Lactate limited	43	0.63	42	0.99
D. SO_4^{2-} limited	39	0.99	39	0.44

Table 5. Glucose inhibition of biofilm formation by wild type *D. vulgaris*

Supplement	Initial culture pH		
	pH 7.0	pH 6.0	pH 5.5
None	4.0	4.6	2.9
100 µM norspermidine	5.0	4.0	3.0
40 mM glucose	3.5	4.4	0.3
100 µM norspermidine plus 40 mM glucose	3.8	1.7	0.3

Biofilm formation monitored by crystal violet staining of material on glass

CONCLUSIONS

- Components of the arginine biosynthetic pathway and polyamine(s) appear to be the major factors effecting the attachment of *D. vulgaris* biofilm material to glass walls and its accumulation on tube bottom.
- Biofilm formation on glass was the best at 30 C. There was no polyamine(s) biofilm stimulation at 37 C.
- Biofilm formation was increased in all three deletion mutants (ΔFur, ΔPerR, and ΔZur) under undisturbed culture conditions. However, only ΔZur accumulated more biofilm on the tube bottom when cultures were agitated.
- Arginine pathway intermediates and polyamines stimulated biofilm formation.
- Nutrient limitation (e.g. ammonium and lactate) enhanced biofilm formation.
- Addition of glucose inhibited biofilm formation, especially on acid media.

ACKNOWLEDGEMENT

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